source of supply for the town all of the fountains save one gave water which was badly contaminated. This fountain, from which the water was excellent, was the first on the pipe coming from the springs, or the one nearest the source of supply, higher than and perhaps a quarter of a mile from the next fountain on this same pipe line. Samples from the springs showed the water to be uniformly excellent. Samples taken on the two succeeding days confirmed the first analyses. Fountains designated by the patients as those from which they had drunk were all on the supply line from which the water was contaminated.

These analyses indicated a point of contamination between the first and second fountains on the main supply line. Search revealed a break in one joint of the pipe. This joint was directly behind, 15 feet from and 12 feet lower than a latrine dug into the downward sloping bank at the edge of the road. The latrine had been used for three months by passing troops, and had been filled with earth on August 27. A lead pipe which supplied the second fountain on this line was set into the main supply line about 30 feet above the break and ran diagonally up the bank, passing directly behind the latrine and under the road. Steps leading to the latrine exposed this pipe at a point about 6 feet beyond and 2 feet higher than the top of the latrine. Here the lead pipe had been cut and was flowing about 3 gallons per minute, thus causing the earth around the latrine and between it and the main pipe to be thoroughly saturated with water. Fecal matter, practically fresh and undecomposed, was found in veins running about and over the main supply pipe. The main supply pipe was repaired and the condition finally corrected on September 6.

Military reasons made it necessary for the writer to leave this section about a week later. At the time of departure all of the patients had recovered or were doing well. Reports from the secretary of the town showed that no new cases developed later in September or during October, nor were there any further deaths.

CONCLUSIONS

The causative factor of the epidemic reported was B. dysenteriae of the Flexner type.

The principal carrier of infection in the above epidemic was water from the main source of supply for the town of Bertrichamp.

ON THE PROTECTION AGAINST THE ACTION OF ULTRAVIOLET LIGHT AFFORDED TO ALEXIN AND SENSITIZER BY CERTAIN SUBSTANCES *

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That ultraviolet light possesses a highly destructive action on various immune bodies — alexin, lysins, agglutinins, toxins and antitoxins, as well as on protoplasm in general, when these substances are exposed either alone or together with certain fluorescent substances[1] such as eosin and fluorescein, has been known for some time. The investigations of Huber[2] and Busck,[3] and particularly those by Baroni and Jonesco-Mihaiesti[4] and by Abelin and Stiner[5] with immune substances bear out this statement. The results indicate that alexin is particularly susceptible to the action of ultraviolet light, and that the time required for complete destruction varies with the thickness of the layer exposed and the serum dilution. More recently the ground has been gone over again by Bovie,[6] Brooks,[7] and Sellards[8] in studying the action of tropical sunlight and results obtained which are essentially in accord with the earlier work.

It has also been noted that the action of ultraviolet light may be inhibited by the addition to the photosensitive substance of blood serum, egg white[9] and various other protein substances, or when the rays are first passed through a glass instead of a quartz vessel. This

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[1] Ledoux-Lebard: Annal. l'Inst. Past., 1902, 16, p. 587; Raab, O.: Ztschr. f. Biol., 1900, 39, p. 524; Raab, O.: Ztschr. f. Biol., 1902, 44, p. 16; von Tappeiner, H., and Jodlbauer, A.: München. med. Wchnschr., 1904, 51, p. 737 and 1139; von Tappeiner, H., and Jodlbauer, A.: Deutsch. Arch. f. klin. Med., 1904, 80, p. 427; Lichtwitz, L.: München. med. Wchnschr., 1904, 51, p. 1589; Pfeiffer, H.: Wien klin. Wchnschr., 1905, 18, pp. 221 and 328; Sacharoff, G., and Sachs, H.: München. med. Wchnschr., 1905, 52, p. 297; Fleischmann, P.: München. med. Wchnschr., 1905, 52, p. 693; Hasselbalch, K. A.: Biochem. Ztschr., 1909, 19, p. 435.

[2] Arch. f. Hyg., 1905, 54, p. 53.

[3] Biochem. Ztschr., 1906, 1, p. 425.

[4] Compt. rend. Soc. de biol., 1910, 68, p. 393.

[5] Ztschr. f. Immunitätsforsch. u. exp. Therap., 1913, 19, p. 1.

[6] Jour. Med. Research, 1918, 38, p. 335.

[7] Jour. Med. Research, 1918, 38, p. 345.

[8] Jour. Med. Research, 1918, 38, p. 293.

[9] Henri, V., and Wurmser, R.: Compt. rend. Soc. de biol., 1912, 73, p. 319.

protective action is due to the absorption of the ultraviolet rays by the protecting agent. Soret[10] has shown that most proteins exhibit an absorption band in the ultraviolet end of the spectrum and that solutions of tyrosin exhibit this phenomenon most markedly. Kober[11] carried out spectrographic investigations on various amino-acids and split products of protein hydrolysis, and found that with the exception of the aromatic amino-acids, tyrosin and phenylalanin, the amino-acids, like the aliphatic acids, esters and certain other compounds investigated by Bielecki and Henri,[12] show no specific absorption. In the instance of the two mentioned aromatic amino-acids, the absorption is very marked and leads one to believe that the protective action afforded by certain proteins is due to their content of these amino-acids.

Harris and Hoyt,[13] working in this laboratory, have confirmed these general results by studying the protection afforded to paramecia and certain bacteria by tyrosin and several other substances against the toxic action of ultraviolet light. It was this work that led to the present investigation which has for its object a more detailed study of aromatic substances as protecting agents against ultraviolet light. To measure this action we have chosen to work with alexin and the sensitizer for sheep cells, components of the well known hemolytic system, since these substances are easily measured, are very susceptible to the action of ultraviolet light, and exhibit moreover a difference in susceptibility toward these rays as well as toward heat. By determining the amount of either alexin or sensitizer to just cause complete hemolysis of the sheep cells, the amount of destruction due to exposure to the light as well as the protection afforded by various substances can be easily determined.

As a source of ultraviolet light we employed a Cooper-Hewitt—type Z—quartz mercury arc lamp, the tube being about 12 cm. above the solution to be tested for protective power. The latter was measured into quartz beakers mounted on black cardboard so that only the light passing through this solution could act on the serum contained in a shallow glass dish packed in ice. In the experiments on alexin, pooled guinea-pig serums were used in a dilution of 1:10 and sensitizer standardized in terms of alexin (0.1 c c), two units of the latter being used for the hemolytic system. In carrying out experiments with sensitizer, two serums were used, designated as A (1:9,500) and B (1:2,000) and the dilutions kept constant as originally standardized against alexin.

[10] Arch. d. Sc. phys. et nat., Geneva, 1878, p. 322, and 1883, p. 194.

[11] Jour. Biol. Chem., 1915, 22, p. 433.

[12] Compt. rend. Acad. d. sc., 1912, 155, pp. 456, 1617; 1913, 156, pp. 550, 884, 1860; 157, p. 372; Berichte, 1912, 45, p. 2819; 1913, 46, p. 1304. See also, Baly, E. C. C., and Desch, C. H.: Jour. Chem. Soc. London, 1904, 85, p. 1029.

[13] Sc., N. S., 1917, 46, p. 318; Univ. Cal. Pub. Path., 1919, 2, p. 245.

Table 1 shows the time required for destruction of alexin and sensitizer, absence of hemolysis indicating destruction. It will be noted that the unit of sensitizer is more easily destroyed than the unit of alexin, although a longer time is required for the lower (serum B) than for the higher dilution (serum A). The unit of sensitizer is, however, the same in both cases, and it is therefore probable that the protection afforded to B is due to the greater concentration of serum

TABLE 1

(A) SHOWING TIME NECESSARY TO DESTROY ALEXIN AND RABBIT VS. SHEEP CELL SENSITIZER BY EXPOSURE TO ULTRAVIOLET LIGHT

Substance Exposed	Time of Exposure, Minutes	Result Degree of hemolysis is indicated by +, ++, +++. Inhibition of hemolysis indicates destruction of alexin or sensitizer. 0.2 c c each of alexin and sensitizer used in the hemolytic system.				
		0.0 c c	0.2 c c	0.3 c c	0.4 c c	0.5 c c
Alexin 1:10.....	2	—	+++	+++	+++	+++
	4	—	—	—	+	++
	6	—	—	—	—	—
Sensitizer "A" 1:9,500.....	1	—	+++	+++	+++	+++
	2	—	—	—	++	+++
	3	—	—	—	—	—
Sensitizer "B" 1:2,000.....	3	—	—	+	++	++
	4	—	—	—	—	+
	5	—	—	—	—	—
Sensitizer "B" 1:10.....	30	—	—	+	++	++
After exposure diluted 1:2,000 for hemolytic system	45	—	—	—	+	++
	60	—	—	—	—	—

(B) PROTECTION AFFORDED TO ALEXIN AND SENSITIZER BY THE HOMOLOGOUS SERUM (INACTIVATED)

Protecting Substance	Time of Exposure, Minutes	Result				
		0.0 c c	0.2 c c	0.3 c c	0.4 c c	0.5 c c
Alexin 1:10.....	30	—	+	++	+++	+++
	45	—	—	+	++	++
Sensitizer "B" 1:10.....	30	—	—	+	++	++
	45	—	—	—	+	++

proteins. While the unit of sensitizer as compared with that of alexin is apparently more readily destroyed, yet alexin is actually more susceptible to the action of ultraviolet light than sensitizer. The apparent differences are due to dilution. When sensitizer in equivalent dilution (1:10) is exposed to ultraviolet rays 1 hour is required for complete destruction of the unit, while for alexin complete destruction takes place in about 5 minutes. The comparison between alexin and sensi-

tizer in equivalent dilutions is only approximate, since the time for destruction depends on the concentration of serum proteins which in the instance of the rabbit[14] and the guinea-pig[15] is different. Measured in terms of protective action the serums are not markedly different, as shown in the second part of table 1. The protective action of alexin and sensitizer in equivalent dilutions (both having been inactivated at 56 C.) for the homologous immune body was determined. Although alexin has been destroyed, the serum still possesses marked protective action, or in other words, ability to absorb ultraviolet rays.

TABLE 2

Showing Protection of Alexin by Various Substances When Exposed to the Action of Ultraviolet Light

Substance and Concentration	Solvent	Time of Exposure, Minutes	Result Degree of hemolysis is indicated by +, ++, +++. Inhibition of hemolysis indicates destruction of alexin. 0.2 c c alexin = 2 units.				
			0.0 c c	0.2 c c	0.3 c c	0.4 c c	0.5 c c
Glycocoll M/10	N/10 KOH	15	—	—	—	—	++
Alanin M/10	H_2O	15	—	—	—	—	+
Taurin M/10	N/10 KOH	15	—	—	—	—	—
Cystin M/20	N/10 HCl	15	—	—	+	+	++
Tyrosin M/20	N/10 KOH	90	+	++	+++	+++	+++
Phenylalanin M/20	N/40 NH_4OH	90	—	+++	+++	+++	+++
Phenylglycocoll M/20	N/20 KOH	90	—	++	++	+++	+++
Sulphanilic acid M/10	H_2O	90	—	+++	+++	+++	+++
Casein 1%	N/10 KOH	90	—	+++	+++	+++	+++
Gelatin 1%	H_2O	15	—	—	—	—	—
Witte's peptone 1%	H_2O	90	—	+++	+++	+++	+++
Protamin (salmin) sulphate 1%	H_2O	30	—	++	++	+++	+++
		45	—	+	+	++	++
Amino benzoic acid M/10	H_2O	90	—	+++	+++	+++	+++
Sodium benzoate M/10	H_2O	90	—	+++	+++	+++	+++
Benzoic acid M/10	H_2O	90	—	+++	+++	+++	+++
Anilin 1%	H_2O	90	—	+++	+++	+++	+++

We next measured the protective power of various amino-acids, proteins, and certain substances of the aromatic series, the results being summarized in tables 2 and 3. The time of exposure as given is exclusive of the time necessary for destruction of immune body when the solvent alone is used as protecting agent. Proper controls were run to insure uniformity of conditions and destruction of immune body without protecting solution. It will be noted that alanin, glycocoll, taurin, and gelatin have little or no protective action against the destructive action of ultraviolet light. Marked protection is, however, shown by the aromatic amino-acids, tyrosin and phenylalanin and also

[14] Woolsey, J. H.: Jour. Biol. Chem., 1913, 14, p. 433.
[15] Wells, C. E.: Jour. Biol. Chem., 1913, 15, p. 37.

by various substances of the aromatic series. Casein and Witte's peptone both protect. It was expected that protamin (salmin) sulphate, due to its almost complete lack of aromatic amino-acids, would show no protective action; however, on testing our preparation, a positive test for tyrosin was obtained. This was true also for the preparation of cystin used. The results also show that the benzol ring is the determinatve factor and not the groups attached to the ring. Thus anilin, benzoic acid, and sulphanilic acid protect equally well. The protection afforded by casein and the absence of any protective action shown by gelatin is due to the difference of aromatic amino-acid content, gelatin lacking these.

TABLE 3

SHOWING PROTECTION OF RABBIT VS. SHEEP CELL SENSITIZER BY VARIOUS SUBSTANCES WHEN EXPOSED TO THE ACTION OF ULTRAVIOLET LIGHT

Substance and Concentration	Solvent	Time of Exposure, Minutes	Result Degree of hemolysis is indicated by +, ++, +++. Inhibition of hemolysis indicates destruction of alexin. 0.2 c c alexin = 2 units.				
			0.0 c c	0.2 c c	0.3 c c	0.4 c c	0.5 c c
Glycocoll M/10	N/10 KOH	15	—	—	—	—	+
Alanin M/10	H_2O	15	—	—	—	—	—
Taurin N/10	N/10 KOH	15	—	—	—	+	+
Cystin M/20	N/10 HCl	30	—	—	+	+	++
Tyrosin M/20	N/10 KOH	90	—	+++	+++	+++	+++
Phenylalanin M/20	N/40 NH_4OH	90	—	+++	+++	+++	+++
Phenylglycocoll M/20	N/20 KOH	60	—	++	+++	+++	+++
Sulphanilic acid M/10	H_2O	90	—	++	+++	+++	+++
Casein 1%	N/10 KOH	90	—	+++	+++	+++	+++
Gelatin 1%	H_2O	15	—	+	+	++	+++
		30	—	—	—	—	—
Witte's peptone 1%	H_2O	90	—	++	++	+++	+++
Protamin (salmin) sulphate 1%	H_2O	30	—	++	+++	+++	+++
		45	—	—	+	++	++
Amino benzoic acid M/10	H_2O	90	—	+++	+++	+++	+++
Sodium benzoate M/10	H_2O	90	—	+++	+++	+++	+++
Benzoic acid M/10	H_2O	90	—	+++	+++	+++	+++
Anilin 1%	H_2O	90	—	+++	+++	+++	+++

Note: In the first three determinations sensitizer "A" (1:9,500) was used. In the others sensitizer "B" (1:2,000) was used.

It is a fundamental concept in photochemistry that action by light cannot take place without absorption. Since absorption in proteins is largely due to the content of aromatic amino-acids it would seem to follow that in the case of substances such as the immune bodies, destruction is due to the content of aromatic amino-acids, unless the action be secondary as in the instance of leucin noted by Harris and Hoyt.[13] It is possible that the difference in susceptibility shown by alexin and sensitizer is due to a difference in aromatic amino acid content.